*Aquatic Mammals* 2008, *34*(2), 157-165, DOI 10.1578/AM.34.2.2008.157

# Evaluation of Aqueous Humor as a Surrogate for Serum Biochemistry in California Sea Lions (*Zalophus californianus*)

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**Abstract**

The aim of this study was to evaluate the use of *post mortem* aqueous humor analysis in estimating serum chemistry in California sea lions (*Zalophus californianus*). Serum and aqueous humor from the left eye were collected from 35 sea lions that were euthanized due to poor prognosis. Each animal was examined *post mortem*, and each head was preserved at ambient temperature. Aqueous humor was collected from the right eye either 24 or 48 h after death. All samples were analyzed with an automated chemistry analyzer by bichromatic photometry and potentiometry. Blood urea nitrogen, creatinine, sodium, chloride, and magnesium showed significant positive linear relationships between serum and aqueous humor values both at the time of death and 24 h later. For aqueous humor sampled after 48 h, the relationships were only significant for blood urea nitrogen and creatinine. For the remaining chemistry parameters, the relationship between levels of those from serum and in aqueous humor were not significant. Serum and aqueous humor collected from 15 animals diagnosed with renal failure were evaluated for antibodies to *Leptospira* using the microscopic agglutination test. All tested sera were positive for *L. interrogans serovar pomona*, with titers greater than 1:25,600. Of titers tested in the aqueous humor, 56% were positive, having values of 1:200 to 1:12,800. These results indicate that an accurate estimate of serum blood urea nitrogen and creatinine can be made from the analysis of aqueous humor at necropsy of California sea lions within 48 h of death, facilitating the diagnosis of renal failure in beached carcasses (one of the most common causes of death in wild sea lions).

**Key Words:** aqueous humor, serum biochemistry, California sea lion, *Zalophus californianus*

## Introduction

Strandings of California sea lions (*Zalophus californianus*) have increased over the past 25 y in California (Greig et al., 2005). This parallels the increase in the wild sea lion population and also may indicate a greater awareness of strandings by the public and of organized stranding networks (Gulland et al., 2001a). Pathology data from these strandings can provide useful information regarding epizootics, environmental contaminants, and anthropogenic-associated morbidity and mortality. The most common causes of sea lion strandings are malnutrition, leptospirosis, trauma, domoic acid toxicosis, and cancer (Greig et al., 2005). Diagnosis of these conditions can be challenging because it often is dependent upon combining clinical signs, blood chemistry (Bossart et al., 2001), and histopathology. Stranded animals often are autolyzed, and if autolysis is severe, histopathology is less informative. Also, by histopathology alone, it is not always possible to determine whether renal lesions translate into the presence or absence of renal failure. Lastly, when working with marine wildlife, *ante mortem* clinical history, signs, or hematology and serum chemistry values often are unavailable to the pathologist. After death, blood rapidly deteriorates due to *post mortem* blood clotting, contamination by bacteria, release of intracellular chemicals, and metabolism of serum compounds.

In humans and domestic animals, analysis of eye fluid collected *post mortem* has been used to estimate *ante mortem* serum chemistry values, drug and toxin levels, and time of death (Wilkie & Bellamy, 1982; Hanna et al., 1990; Berkowicz et al., 2001; Jones & Holmgren, 2001; Brzezinski & Godlewski, 2004). Eye fluid is composed of the vitreous and aqueous humors. The vitreous humor is a clear gel that is located in the posterior compartment of the eye between the lens and the retina and occupies up to 80% of the volume of the eyeball. It is composed mainly of water (99%), inorganic salts (0.9%), collagen and hyaluronic

acid (0.1%), hyalocytes, sugar, and ascorbic acid (Swann et al., 1975). The aqueous humor is a thin, watery fluid filling the anterior chamber of the eye. It is produced continually by the ciliary body (by secretion, diffusion, and ultrafiltration of the bloodstream), then flows into the anterior chamber through the pupil, exits through the trabecular meshwork located in the drainage angle, and returns to the bloodstream through the Schlemm's canal (Johnson & Kamm, 1983). As both vitreous and aqueous humor are protected anatomically, they undergo minimal *post mortem* change.

In forensic medicine especially, the analysis of the vitreous humor as a surrogate for serum traditionally is preferred to aqueous humor (Coe, 1972). Currently, analytic technology allows determination of a wide range of chemistry parameters in a relatively low volume of fluid. This development now enables the use of aqueous humor rather than vitreous humor as a surrogate for serum in domestic animals (Hanna et al., 1990). The use of aqueous humor has several advantages to vitreous humor. It is easier to collect and thus reduces the potential for sample contamination, such as with blood, and it requires less handling (filtering) prior to analysis.

The purpose of this study was to determine whether evaluation of biochemical parameters in the aqueous humor could be used as a surrogate for serum biochemistry in California sea lions. The objectives were to evaluate biochemistry values and antibody titers for *Leptospira* spp. in the aqueous humor collected at time of death and either 24 h (T24) or 48 h (T48) after death and to compare them to *ante mortem* values.

## Materials and Methods

*Subjects*

All of the California sea lions in the study ($n$ = 35) stranded along the California coast (between 37º 42' N, 123º 05' W and 35º 59' N, 121º 30' W) from October 2006 to February 2007 and were transported to The Marine Mammal Center in Sausalito, California, for rehabilitation. These sea lions (26 males, 9 females) were euthanized due to poor prognosis (Gulland et al., 2001b). Body weight ranged from 13 to 168 kg, and age class ranged from yearling to adult. Of the 35 sea lions sampled, 15 were diagnosed with renal failure and leptospirosis based on clinical signs and/or necropsy results: serum chemistry showed a blood urea nitrogen > 100 mg/dL, creatinine > 2 mg/dL, and phosphorus > calcium; gross necropsy revealed pale, swollen kidneys with loss of cortico-medullary differentiation; and histopathology revealed tubulointerstitial nephritis (Gulland et al., 1996; Colagross-Schouten et al., 2002). The other 20 animals involved in the study suffered malnutrition, trauma, domoic acid toxicosis, and cancer as defined in Greig et al. (2005). Animal selection included those with a variety of diseases to ensure a wide range of serum biochemistry parameters.

*Sample Collection*

All animals were sedated with a 1:1 solution of tiletamine and zolazepam (1 mg/kg or more if necessary) (Telazol, Fort Dodge Animal Health, Fort Dodge, Iowa, USA) administered intramuscularly. Once sedated, blood was drawn from the subclavian vein using an 18 G 3.8-cm needle directly into a vacutainer containing serum separation gel (Vacutainer SST gel clot activator, Becton Dickinson, NJ, USA). All blood samples were obtained within 1 min of euthanasia (T0). Subsequently, all animals were euthanized by injection of pentobarbital (80 mg/kg Beuthanasia, Schering-Plough Animal Health Corporation, Kenilworth, NJ, USA) into the subclavian vein, generally using the same site as for blood collection. Aqueous humor was obtained from the left eye within 5 min of death (T0) by a trans-scleral approach at the limbus using a sterile 6-ml syringe and a 20 G 2.5-cm needle. Approximately 2 ml of aqueous humor was extracted. Samples that were heavily pigmented or obviously contaminated with blood were excluded from the study. Each animal was examined *post mortem*, and each head was preserved at ambient temperature (4 to 20° C) in a clean, open plastic container. The eye that was to be sampled at T24 or T48 remained in the skull following necropsy to simulate natural conditions. Care was taken during handling to maintain the integrity of the periorbital tissues. Aqueous humor was collected from the right eye either at T24 (Group A, $n$ = 20) or T48 (Group B, $n$ = 15). Following sampling, both eyes were removed and preserved in Davidson's fixative for 3 d and then transferred into formalin for future histological examination.

*Sample Processing*

Within 30 min of collection, aqueous samples were transferred into cryovials, and blood samples were centrifuged (2,000 rotations/min) for 15 min for serum separation. Serum and aqueous samples were stored at 4° C and analyzed within 24 h of collection using an automated chemistry analyzer (Alfa Wasserman Vet Ace, 4 Henderson Drive, West Caldwell, NJ, USA), and the remaining serum was archived at -80° C. The following parameters were measured: total iron, cholesterol, triglycerides, gamma glutamyl transferase (GGT), alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALK), total bilirubin (TBili), glucose, phosphorus, total protein (TP), blood urea nitrogen (BUN), creatinine, calcium, sodium, potassium, chloride, magnesium, albumin, and creatine

kinase (CK). All chemistry concentrations were measured by bichromatic photometry except for electrolytes (i.e., sodium, potassium, and chloride), which were determined by potentiometry. Serum and aqueous humor collected from 15 animals (9 from Group A and 6 from Group B) diagnosed with leptospirosis were evaluated for antibodies to six *Leptospira* serovars using the Microscopic Agglutination Test (Oklahoma Animal Disease Diagnostic Lab Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK, USA). All the aqueous humor samples tested for *Leptospira* antibodies also were checked for the presence of hemoglobin using an automated hematology analyzer (Vet ABC Heska) to preclude contamination with blood-derived antibodies as a cause of seropositivity.

*Statistical Analysis*

Mean and SD were calculated for each parameter in the serum and aqueous humor. After removing all zeroes from the dataset, linear least squares regression analysis was used to model the relationship between biochemical parameters in *ante mortem* serum and *post mortem* aqueous humor collected at T0, T24, and T48. Both the slope of the linear relationship and the variability around the line were evaluated for each chemistry parameter. The relationship between serum and aqueous humor was considered to be of diagnostic interest if there was a significant positive linear relationship ($p < 0.05$) with the slope of the line approaching 1 and a Pearson's correlation coefficient $R^2$ greater than 0.80. Descriptive statistics and regression analyses were performed using *SPSS*, Version 11.0 (Chicago, IL, USA).

## Results

Histological examination of 10 eyes (two eyes collected on five animals) following sampling showed no lesions associated with disease or artifacts from the collection method.

All serum ($n = 35$) and aqueous humor ($n = 70$) samples were of adequate quality and volume for analysis and banking, thus a total of 105 samples were processed. For unknown technical reasons, some biochemical analyte concentrations could not be determined in all of the samples: total iron concentration could not be measured in one serum and one aqueous humor sample, triglycerides in one serum sample, GGT in one serum sample, AST concentration in 18 aqueous humor samples, ALK in one serum sample, BUN in one aqueous humor sample, creatinine in one serum and one aqueous humor sample, potassium in two aqueous humor samples, magnesium in four aqueous humor samples, albumin in one aqueous humor sample, and CK in three serum and eight aqueous humor samples.

Serum biochemical parameters revealed a range of values that were similar to some of those observed in aqueous humor (Tables 1, 2 & 3). For glucose, BUN, and creatinine, there was a significant positive linear relationship between serum and aqueous humor at T0 (Table 1). These relationships held at T24 and at T48 with the exception of glucose (Tables 2 & 3). Among the Group A animals, aqueous humor values for sodium, chloride, and magnesium at T0 and T24 were predictive of serum values at T0. When the T0 values from both groups were combined (Table 1), these relationships did not hold suggesting that the T24 results may not be useful diagnostically. A serum BUN concentration greater than 100 mg/dL usually is considered indicative of renal failure; therefore, as demonstrated in Figure 1, even when BUN values were greater than 100 mg/dL, the aqueous humor value was predictive of the serum *ante mortem* value. The same was true for creatinine values greater than 2 mg/dL as presented in Figure 2. For the remainder of the parameters, the aqueous humor chemistry values were not predictive of serum chemistry values.

The concentration of glucose in the aqueous humor collected at T24 or T48 always was extremely low (≤ 3 mg/dL), except for animals with marked *ante mortem* hyperglycemia. In contrast, the aqueous humor concentrations of other analytes, such as potassium, phosphorus, AST, and CK, were greatest at T48.

All sera tested for *Leptospira* antibody titers ($n = 15$) were positive for *L. interrogans serovar pomona* and were ≥ 1:102,400, except for one case (1:25,600). Of titers tested in aqueous humor ($n = 30$), 13 were negative and 17 were positive having values of 1:200 to 1:12,800. Hemoglobin was not found in any of the aqueous humor samples, which precludes contamination of the aqueous humor with whole blood.

## Discussion

This study demonstrates that aqueous humor can be collected from dead animals and used as a surrogate for serum to predict *ante mortem* serum chemistry values for several parameters in California sea lions. Although only a limited number of parameters in aqueous humor were predictive of serum values, these parameters, namely BUN, electrolytes, and creatinine, commonly are altered in renal disease; therefore, analysis of aqueous humor may assist in diagnosis of renal disease in sea lions. Furthermore, antibodies to leptospirosis, which is the most common cause of renal disease and infectious mortality in stranded California sea lions (Greig et al., 2005), were

**Table 1.** Mean ± SD concentrations of biochemical parameters in serum and aqueous humor of California sea lions at the time of death (T0); values in bold have a significant positive linear relationship between serum and aqueous humor ($p < 0.05$ and $R^2 > 0.80$), and ns = not significant at the $p < 0.05$ level.

| | Concentration in serum at T0 | | | Concentration in aqueous humor at T0 | | | Correlation |
|---|---|---|---|---|---|---|---|
| Biochemical parameter | *n* | Mean ± SD | Range | *n* | Mean ± SD | Range | $R^2$, *p value* |
| Total iron (µg/dL) | 34 | 106.0 ± 44.0 | 26.0-179.0 | 34 | 42.0 ± 52.0 | 0.0-161.0 | ns |
| Cholesterol (mg/dL) | 35 | 224.0 ± 89.0 | 75.0-460.0 | 35 | 5.0 ± 7.0 | 2.0-35.0 | ns |
| Triglycerides (mg/dL) | 35 | 204.0 ± 258.0 | 17.0-1,275.0 | 35 | 11.0 ± 5.0 | 5.0-25.0 | ns |
| γGlutamyl transferase (U/L) | 34 | 228.0 ± 179.0 | 51.0-915.0 | 35 | 7.0 ± 19.0 | 0.0-109.0 | $R^2 = 0.13$, $p = 0.038$ |
| Alanine transaminase (U/L) | 35 | 72.0 ± 46.0 | 10.0-203.0 | 35 | 17.0 ± 5.0 | 1.0-29.0 | ns |
| Aspartate transaminase (U/L) | 35 | 76.0 ± 116.0 | 4.0-609.0 | 21 | 19.0 ± 18.0 | 3.0-63.0 | ns |
| Alkaline phosphatase (U/L) | 34 | 51.0 ± 36.0 | 12.0-202.0 | 35 | 2.0 ± 3.0 | 0.0-15.0 | ns |
| Total bilirubin (mg/dL) | 35 | 0.8 ± 0.8 | 0.2-4.0 | 35 | 0.7 ± 0.6 | 0.1-2.5 | ns |
| **Glucose (mg/dL)** | **35** | **134.0 ± 93.0** | **2.0-464.0** | **35** | **127.0 ± 74.0** | **24.0-375.0** | **$R^2 = 0.82$, $p < 0.001$** |
| Phosphorus (mg/dL) | 35 | 11.2 ± 6.4 | 0.6-24.9 | 35 | 5.6 ± 4.7 | 0.4-18.2 | $R^2 = 0.47$, $p < 0.001$ |
| Total protein (g/dL) | 35 | 8.5 ± 1.2 | 5.7-10.7 | 35 | 0.5 ± 0.6 | 0.0-2.4 | ns |
| **Blood urea nitrogen (mg/dL)** | **35** | **164.0 ± 160.0** | **14.0-510.0** | **34** | **176.0 ± 175.0** | **14.0-534.0** | **$R^2 = 0.97$, $p < 0.001$** |
| **Creatinine (mg/dL)** | **34** | **5.3 ± 7.2** | **0.3-28.1** | **35** | **3.7 ± 5.1** | **0.0-19.9** | **$R^2 = 0.94$, $p < 0.001$** |
| Ca (mg/dL) | 35 | 8.4 ± 0.9 | 5.7-9.5 | 35 | 6.2 ± 1.2 | 0.6-8.0 | ns |
| Na (mmol/L) | 35 | 159.0 ± 15.0 | 138.0-199.0 | 35 | 148.0 ± 21.0 | 61.0-183.0 | $R^2 = 0.55$, $p < 0.001$ |
| K (mmol/L) | 35 | 5.0 ± 1.4 | 2.4-8.2 | 34 | 5.8 ± 1.7 | 3.3-10.7 | $R^2 = 0.15$, $p = 0.026$ |
| Cl (mmol/L) | 35 | 119.0 ± 17.0 | 98.0-186.0 | 35 | 120.0 ± 16.0 | 99.0-154.0 | $R^2 = 0.68$, $p < 0.001$ |
| Mg (mg/dL) | 35 | 3.3 ± 1.6 | 1.3-7.9 | 32 | 1.8 ± 1.2 | 0.1-5.5 | $R^2 = 0.72$, $p < 0.001$ |
| Albumin (g/dL) | 35 | 2.8 ± 0.5 | 1.5-3.7 | 34 | 0.4 ± 0.8 | 0.0-4.9 | ns |
| Creatine kinase (U/L) | 32 | 1,040.0 ± 2,290.0 | 67.0-13,045.0 | 32 | 25.0 ± 54.0 | 0.0-255.0 | ns |

**Table 2.** Mean ± SD concentrations of biochemical parameters in serum and aqueous humor of California sea lions at the time of death (T0) and 24 h after death (T24); values in bold have a significant positive linear relationship between serum and aqueous humor at T0 and T24 with $p < 0.05$ and $R^2 > 0.80$. Glucose was the only analyte that was significant at T0 but not at T24. The $p$ value and $R^2$ describe the relationship between serum at T0 and aqueous humor at T24.

| | Concentration in serum at T0 | | | Concentration in aqueous at T0 | | | Concentration in aqueous humor at T24 | | | Correlation |
|---|---|---|---|---|---|---|---|---|---|---|
| Biochemical parameter | *n* | Mean ± SD | Range | *n* | Mean ± SD | Range | *n* | Mean ± SD | Range | $R^2$, *p value* |
| Total iron (µg/dL) | 20 | 110.0 ± 42.0 | 41.0-179.0 | 19 | 66.0 ± 52.0 | 2.0-161.0 | 20 | 25.0 ± 28.0 | 1.0-89.0 | ns |
| Cholesterol (mg/dL) | 20 | 230.0 ± 100.0 | 75.0-460.0 | 20 | 6.0 ± 10.0 | 2.0-35.0 | 20 | 8.0 ± 14.0 | 3.0-64.0 | ns |
| Triglycerides (mg/dL) | 20 | 255.0 ± 313.0 | 26.0-1,275.0 | 20 | 11.0 ± 6.0 | 6.0-25.0 | 20 | 31.0 ± 12.0 | 8.0-50.0 | ns |
| γGlutamyl transferase (U/L) | 20 | 223.0 ± 145.0 | 51.0-577.0 | 20 | 10.0 ± 25.0 | 1.0-109.0 | 20 | 11.0 ± 20.0 | 0.0-75.0 | ns |
| Alanine transaminase (U/L) | 20 | 66.0 ± 38.0 | 20.0-147.0 | 20 | 17.0 ± 5.0 | 11.0-29.0 | 20 | 12.0 ± 5.0 | 10.0-33.0 | ns |
| Aspartate transaminase (U/L) | 20 | 82.0 ± 135.0 | 4.0-609.0 | 15 | 21.0 ± 19.0 | 5.0-63.0 | 18 | 20.0 ± 32.0 | 4.0-144.0 | ns |
| Alkaline phosphatase (U/L) | 20 | 58.0 ± 42.0 | 12.0-202.0 | 20 | 3.0 ± 3.0 | 0.0-15.0 | 20 | 4.0 ± 3.0 | 0.0-13.0 | ns |
| Total bilirubin (mg/dL) | 20 | 0.8 ± 0.8 | 0.2-4.0 | 20 | 0.8 ± 0.6 | 0.1-2.5 | 20 | 0.6 ± 0.4 | 0.1-1.5 | ns |
| Glucose (mg/dL) | 20 | 124.0 ± 104.0 | 2.0-464.0 | 20 | 125.0 ± 72.0 | 29.0-306.0 | 20 | 13.0 ± 31.0 | 0.0-106.0 | ns |
| Phosphorus (mg/dL) | 20 | 12.0 ± 6.0 | 4.0-24.9 | 20 | 7.0 ± 5.1 | 1.7-18.2 | 20 | 8.9 ± 4.2 | 2.8-18.5 | ns |
| Total protein (g/dL) | 20 | 8.8 ± 1.2 | 6.4-10.7 | 20 | 0.7 ± 0.7 | 0.0-2.4 | 20 | 0.6 ± 0.9 | 0.0-4.0 | ns |
| Blood urea nitrogen (mg/dL) | 20 | **203.0 ± 175.0** | **14.0-510.0** | **20** | **214.0 ± 192.0** | **14.0-534.0** | **20** | **217.0 ± 191.0** | **14.0-510.0** | **$R^2 = 0.99$, $p < 0.001$** |
| Creatinine (mg/dL) | 20 | **6.9 ± 8.3** | **0.3-28.1** | **20** | **5.0 ± 6.1** | **0.3-19.9** | **20** | **4.7 ± 5.8** | **0.2-18.9** | **$R^2 = 0.94$, $p < 0.001$** |
| Ca (mg/dL) | 20 | 8.4 ± 1.0 | 5.7-9.5 | 20 | 6.2 ± 0.7 | 4.4-7.4 | 20 | 7.1 ± 0.7 | 5.1-8.4 | ns |
| Na (mmol/L) | 20 | **158.0 ± 15.0** | **138.0-189.0** | **20** | **149.0 ± 16.0** | **129.0-183.0** | **20** | **148.0 ± 14.0** | **127.0-175.0** | **$R^2 = 0.93$, $p < 0.001$** |
| K (mmol/L) | 20 | 5.2 ± 1.5 | 2.4-8.1 | 20 | 6.2 ± 1.8 | 4.2-10.7 | 20 | 11.8 ± 1.7 | 7.7-14.6 | ns |
| Cl (mmol/L) | 20 | **117.0 ± 13.0** | **98.0-146.0** | **20** | **118.0 ± 17.0** | **99.0-154.0** | **20** | **121.0 ± 15.0** | **106.0-152.0** | **$R^2 = 0.94$, $p < 0.001$** |
| Mg (mg/dL) | 20 | **3.6 ± 1.8** | **1.6-7.9** | **20** | **2.0 ± 1.3** | **0.5-5.5** | **20** | **2.6 ± 1.2** | **1.1-5.6** | **$R^2 = 0.88$, $p < 0.001$** |
| Albumin (g/dL) | 20 | 2.8 ± 0.6 | 1.5-3.7 | 20 | 0.3 ± 0.3 | 0.1-1.3 | 20 | 0.3 ± 0.4 | 0.0-1.8 | ns |
| Creatine kinase (U/L) | 18 | 1,470.0 ± 2,994.0 | 67.0-13,045.0 | 17 | 31.0 ± 59.0 | 2.0-255.0 | 15 | 270.0 ± 752.0 | 15.0-2,980.0 | ns |

**Table 3.** Mean ± SD concentrations of biochemical parameters in serum and aqueous humor of California sea lions at time of death (T0) and 48 h after death (T48); values in bold have a significant positive linear relationship between serum and aqueous humor at T0 and T24 with $p < 0.05$ and $R^2 > 0.80$. Glucose was the only analyte that was significant T0 but not at T24. The $p$ value and $R^2$ describe the relationship between serum at T0 and aqueous humor at T48.

| | Concentration in serum at T0 | | | Concentration in aqueous humor at T0 | | | Concentration in aqueous humor at T48 | | | Correlation |
|---|---|---|---|---|---|---|---|---|---|---|
| Biochemical parameter | *n* | Mean ± SD | Range | *n* | Mean ± SD | Range | *n* | Mean ± SD | Range | $R^2$, *p value* |
| Total iron (µg/dL) | 14 | 100.0 ± 48.0 | 26.0-177.0 | 15 | 12.0 ± 34.0 | 0.0-133.0 | 15 | 18.0 ± 27.0 | 1.0-107.0 | ns |
| Cholesterol (mg/dL) | 15 | 215.0 ± 74.0 | 86.0-375.0 | 15 | 3.0 ± 1.0 | 2.0-6.0 | 15 | 4.0 ± 2.0 | 2.0-10.0 | ns |
| Triglycerides (mg/dL) | 14 | 137.0 ± 143.0 | 17.0-543.0 | 15 | 11.0 ± 5.0 | 5.0-23.0 | 15 | 54.0 ± 20.0 | 30.0-106.0 | ns |
| γGlutamyl tranferase (U/L) | 14 | 234.0 ± 224.0 | 57.0-915.0 | 15 | 3.0 ± 2.0 | 0.0-9.0 | 15 | 4.4 ± 1.8 | 2.0-8.0 | ns |
| Alanine transaminase (U/L) | 15 | 79.0 ± 55.0 | 10.0-203.0 | 15 | 17.0 ± 5.0 | 1.0-24.0 | 15 | 11.0 ± 4.0 | 10.0-26.0 | ns |
| Aspartate transaminase (U/L) | 15 | 67.0 ± 89.0 | 8.0-358.0 | 6 | 13.0 ± 15.0 | 3.0-40.0 | 13 | 16.0 ± 12.0 | 0.0-40.0 | ns |
| Alkaline phosphatase (U/L) | 14 | 41.0 ± 22.0 | 16.0-103.0 | 15 | 1.0 ± 1.0 | 0.0-4.0 | 15 | 2.0 ± 2.0 | 0.0-6.0 | ns |
| Total bilirubin (mg/dL) | 15 | 0.8 ± 0.8 | 0.2-2.7 | 15 | 0.4 ± 0.4 | 0.1-1.7 | 15 | 0.8 ± 0.5 | 0.3-2.0 | ns |
| Glucose (mg/dL) | 15 | 147.0 ± 78.0 | 66.0-411.0 | 15 | 130.0 ± 80.0 | 24.0-375.0 | 15 | 7.0 ± 20.0 | 0.0-69.0 | ns |
| Phosphorus (mg/dL) | 15 | 9.6 ± 6.4 | 0.6-19.2 | 15 | 3.6 ± 3.4 | 0.4-14.1 | 15 | 8.0 ± 3.0 | 4.8-15.0 | ns |
| Total protein (g/dL) | 15 | 8.1 ± 1.2 | 5.7-10.4 | 15 | 0.2 ± 0.2 | 0.0-1.0 | 15 | 0.4 ± 0.6 | 0.1-2.5 | ns |
| Blood urea nitrogen (mg/dL) | **15** | **110.0 ± 124.0** | **18.0-390.0** | **14** | **122.0 ± 136.0** | **21.0-420.0** | **15** | **121.0 ± 141.0** | **15.0-444.0** | **$R^2 = 0.99$, $p < 0.001$** |
| Creatinine (mg/dL) | **14** | **3.1 ± 4.4** | **0.5-15.2** | **15** | **2.0 ± 2.8** | **0.0-8.4** | **14** | **2.5 ± 3.2** | **0.1-10.3** | **$R^2 = 0.99$, $p < 0.001$** |
| Ca (mg/dL) | 15 | 8.3 ± 0.7 | 7.0-9.3 | 15 | 6.1 ± 1.7 | 0.6-8.0 | 15 | 7.3 ± 0.6 | 5.9-8.4 | ns |
| Na (mmol/L) | 15 | 161.0 ± 17.0 | 143.0-199.0 | 15 | 147.0 ± 27.0 | 61.0-177.0 | 15 | 147.0 ± 13.0 | 130.0-170.0 | ns |
| K (mmol/L) | 15 | 4.8 ± 1.2 | 3.0-8.2 | 14 | 5.1 ± 1.2 | 3.3-8.6 | 14 | 15.5 ± 2.9 | 10.8-21.2 | ns |
| Cl (mmol/L) | 15 | 122.0 ± 22.0 | 102.0-186.0 | 15 | 122.0 ± 16.0 | 101.0-145.0 | 15 | 122.0 ± 14.0 | 103.0-143.0 | ns |
| Mg (mg/dL) | 15 | 3.1 ± 1.5 | 1.3-5.0 | 12 | 1.4 ± 0.8 | 0.1-2.6 | 14 | 1.8 ± 0.8 | 0.1-3.2 | ns |
| Albumin (g/dL) | 15 | 2.7 ± 0.5 | 1.6-3.3 | 14 | 0.5 ± 1.3 | 0.0-4.9 | 15 | 0.4 ± 0.7 | 0.1-3.0 | ns |
| Creatine kinase (U/L) | 14 | 488.0 ± 435.0 | 70.0-1,382.0 | 15 | 18.0 ± 48.0 | 0.0-189.0 | 15 | 196.0 ± 203.0 | 2.0-836.0 | ns |

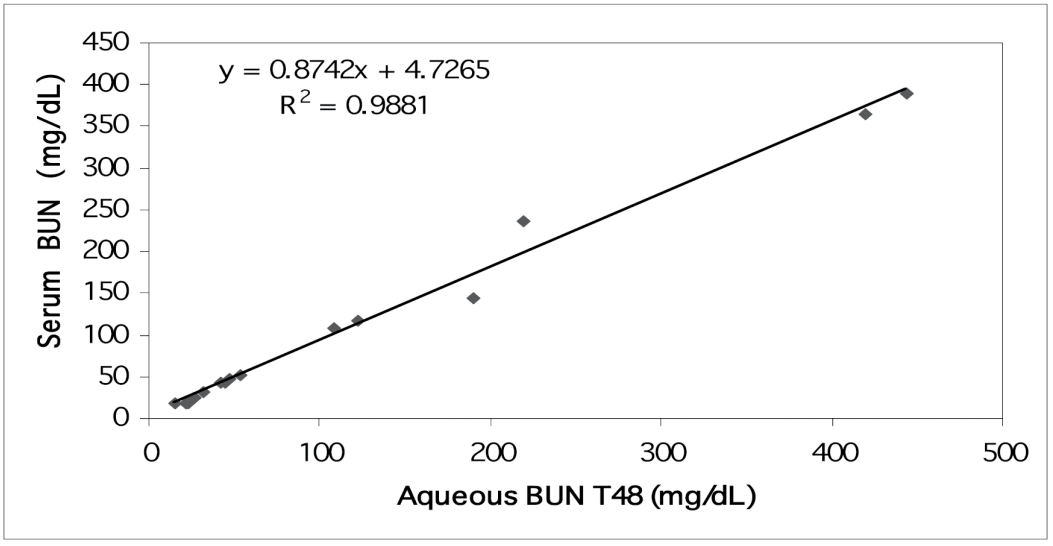


**Figure 1.** The relationship between the BUN concentration in serum sampled at the time of death ($n$ = 20) and in the aqueous humor sampled 48-h *post mortem*

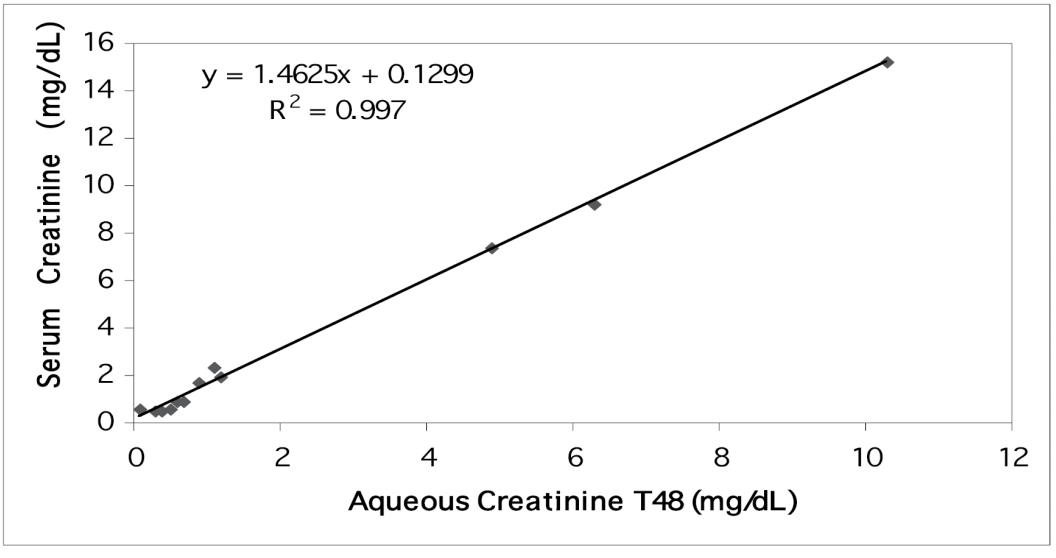


**Figure 2.** The relationship between creatinine concentration in the serum sampled at the time of death ($n$ = 13) and in the aqueous humor sampled at 48-h *post mortem*

detected in aqueous humor in 56% of cases that also had high serum antibody levels.

These findings are consistent with findings in other species, including small and large domestic species, cervids, and West Indian manatees (*Trichechus manatus latirostris*) (Bito et al., 1964; Hanna et al., 1990; Zaugg & Kinsel, 1997; Brzezinski & Godlewski, 2004; McCoy, 2004; Varela & Bossart, 2005). The results suggest that immediately following death there are rapid and pronounced changes in the concentration of most substances in the aqueous humor of sea lions. Similar to domestic species, only a small percentage of large serum molecules, such as cholesterol, bilirubin, and proteins (including enzymes), passed into the aqueous humor. In some aqueous humor samples, the presence of some serum enzymes, such as creatine kinase and AST, likely was associated with their release from the cytoplasm of the

surrounding cells undergoing autolysis or damaged during fluid collection.

In many species, the concentration of BUN and creatinine in aqueous humor approximate *ante mortem* serum values of BUN and creatinine and remain stable at ambient temperatures between 4 and 20° C for up to 48 h after death. Similarly in these sea lions, BUN and creatinine values were predictive of *ante mortem* serum values and remained stable at ambient temperatures up to 48 h after death. In this study, abnormally high serum values for BUN and creatinine ($n$ = 15) were well-represented; and despite an increase in variability at these higher levels, the elevation in serum BUN and creatinine concentrations can be estimated with a high level of confidence from *post mortem* concentrations in aqueous humor. The influence of ambient temperature on these concentrations should be considered because in equine vitreous humor, BUN and creatinine concentrations increase with temperature as well as with time (McLaughlin & McLaughlin, 1988). This study was limited to the effects of ambient temperatures between 4 and 20° C.

Interestingly, in West Indian manatees, fresh and moderately autolyzed carcasses had higher creatinine concentrations in the vitreous humor than the upper limit of the reference range in *ante mortem* serum (Varela & Bossart, 2005); however, creatinine concentrations in the aqueous humor of the sea lions in the current study were all within the range of *ante mortem* serum values. Thus, the site of the collection may influence the comparability between ocular fluid and serum parameters. As another consideration, in cats and cattle, variability in the BUN concentration between the serum and aqueous humor increased when levels were markedly elevated (Hanna et al., 1990). This was attributed to the known lag phase in the equilibration of changing BUN concentrations across the blood-ocular barriers. Some variability in elevated BUN concentrations also was observed in California sea lions.

In this study, several of the electrolyte concentrations did not vary significantly during the first 24 h. In the subset of California sea lions that were dead for less than 24 h, aqueous humor concentrations of sodium, chloride, and magnesium were predictive of the *ante mortem* serum values. In the T48 group and at T0 when both groups were combined, changes in electrolytes were more pronounced and thus the aqueous humor concentrations in sodium, chloride, and magnesium were not predictive of *ante mortem* serum values. These results confirm findings in other studies (Hanna et al., 1990; Gerometta et al., 2005). In most species, the concentration of ions in the aqueous humor differs significantly from that of plasma, increasing with time after death and with increased temperature. It is also important to note that in this study, electrolytes were measured using only potentiometry. Caution should be used when comparing electrolyte concentrations measured with potentiometry to electrolyte concentrations obtained with flame photometry.

The very rapid decrease in glucose concentration demonstrates that glucose utilization by the intraocular tissues continues uninterrupted after death. The present results indicate that for most California sea lions sampled, the glucose initially present in the aqueous humor was eliminated within 24 h after death at ambient temperature. The rate of glucose utilization in the eye varies among domestic species because of morphological differences associated with volume of aqueous humor as compared to volume or surface area of intraocular tissues. Accordingly, glucose concentration in aqueous humor cannot be used for the diagnosis of *ante mortem* hypoglycemia. In contrast, if glucose concentration in aqueous humor collected at least 24 h after death is still relatively high (e.g., > 50 mg/dL), then it is possible to make a tentative diagnosis of *ante mortem* acute or chronic hyperglycemia (Daee et al., 1978; Varela & Bossart, 2005).

*Post mortem* potassium and phosphorus concentrations in the aqueous humor did not correlate well with concentrations in *ante mortem* serum; however, levels in aqueous humor did increase with time. In forensic medicine, the potassium concentration in vitreous humor is used to determine the *post mortem* interval in humans and is dependent on temperature and the ion determination method (Garg et al., 2004). Likewise, in California sea lions, potassium and phosphorus concentrations might potentially be used to estimate *post mortem* interval and degree of autolysis. Further studies are needed to characterize the changes in phosphorus and potassium concentrations with time after death in California sea lions.

All sera tested were positive for *L. interrogans serovar pomona*, which is the most commonly isolated serovar from California sea lions (Colagross-Schouten et al., 2002). The majority of samples from aqueous humor were negative for *Leptospira* antibodies. Hemoglobin was not detected in any sample, suggesting that whole blood contamination was not responsible for the few positive cases. This finding may not preclude serum contamination from autolysis, however, and thus the passive transfer of antibodies from the blood into the aqueous humor *post mortem*. Alternatively, there may have been a compromise of the blood-ocular barrier *ante mortem* for reasons that were not or are no longer apparent on histological examination.

In conclusion, aqueous humor analysis is a promising diagnostic method of assessing

*ante mortem* serum chemistry in California sea lions. Such information will prove useful to personnel examining stranded California sea lions and in diagnosing *ante mortem* renal failure.

**Acknowledgments**

We thank the veterinary technicians and husbandry staff (D. Wickham, A. Makie, and M. Blascow) of The Marine Mammal Center for their constant assistance. The care of stranded marine mammals and the opportunity for this research would not be possible without the many dedicated volunteers at The Marine Mammal Center. We also thank Shirley Sichel for generously funding this project. The work described in this paper was conducted under a Letter of Authorization from the National Marine Fisheries Service's Marine Mammal Health and Stranding Program, and samples were collected under U.S. Marine Mammal Protection Act Scientific Research Permit No. 932-1489-00.

**Literature Cited**

Berkowicz, A., Wallerstedt, S., Wall, K., & Denison, H. (2001). Carbohydrate-deficient transferring in vitreous humor: A marker of possible withdrawal-related death in alcoholics. *Alcohol and Alcoholism*, *36*(3), 231-234.

Bito, L. Z., & Salvador, E. V. (1964). IOF dynamics. II. Post mortem changes. *Experimental Eye Research*, *3*, 274-287.

Bossart, G. D., Reidarson, T. H., Dierauf, L. A., & Duffield, D. A. (2001). Clinical pathology. In L. Dierauf & F. M. D. Gulland (Eds.), *CRC handbook of marine mammal medicine* (pp. 383-430). Boca Raton, FL: CRC Press. 1,063 pp.

Brzezinski, P. M., & Godlewski, A. (2004). Assessment of potassium and sodium ion concentrations in the vitreous humour of swine isolated eyeballs after organism death. *Annales Academiae Medicae Bialostocensis*, *49*(1), 161-163.

Coe, J. I. (1972). Use of chemical determinations on vitreous humor in forensic pathology. *Journal of Forensic Science*, *17*, 541-546.

Colagross-Schouten, A. M., Mazet, J. A. K., Gulland, F. M. D., Miller, M. A., & Hietala, S. (2002). Diagnosis and seroprevalence of leptospirosis in California sea lions from coastal California. *Journal of Wildlife Disease*, *38*(1), 7-17.

Daee, L. N. W., Teige, B., & Svaar, H. (1978). Determination of glucose in human vitreous humor. *Z Rechtsmedizin*, *80*, 287-291.

Garg, V., Oberoi, S. S., Gorea, R. K., & Kaur, K. (2004). Changes in the levels of vitreous potassium with increasing time since death. *Journal of the Indian Academy of Forensic Medicine*, *26*(4), 136-139.

Gerometta, R. M., Malgor, L. A., Vitalta, E., Leiva, J., & Candia, O. A. (2005). Cl concentrations of bovine, porcine and ovine aqueous humor are higher than in plasma. *Experimental Eye Research*, *80*, 307-312.

Greig, D. J., Gulland, F. M. D., & Creuder, C. (2005). A decade of live California sea lion (*Zalophus californianus*) strandings along the central California coast: Causes and trends, 1991-2000. *Aquatic Mammals*, *31*(1), 11-22.

Gulland, F. M. D., Dierauf, L. A., & Rowles, T. K. (2001a). Marine mammal stranding networks. In L. Dierauf & F. M. D. Gulland (Eds.), *CRC handbook of marine mammal medicine* (pp. 45-67). Boca Raton, FL: CRC Press. 1,063 pp.

Gulland, F. M. D., Haulena, M., & Dierauf, L. A. (2001b). Seals and sea lions. In L. Dierauf & F. M. D. Gulland (Eds.), *CRC handbook of marine mammal medicine* (pp. 907-926). Boca Raton, FL: CRC Press. 1,063 pp.

Gulland, F. M. D., Koski, M., Lowenstine, L. J., Colagross, A., Morgan, L., & Spraker, T. (1996). Leptospirosis in California sea lions (*Zalophus californianus*) stranded along the central California coast, 1981-1994. *Journal of Wildlife Disease*, *32*(4), 572-580.

Hanna, P. E., Bellamy, J. E. C., & Donald, A. (1990). Postmortem eyefluid analysis in dogs, cats and cattle as an estimate of antemortem serum chemistry profiles. *Canadian Journal of Veterinary Research*, *54*, 487-494.

Johnson, M. C., & Kamm, R. D. (1983). The role of Schlemm's canal in aqueous outflow from the human eye. *Investigative Ophthalmology and Visual Science*, *24*, 320-325.

Jones, A. W., & Holmgren, P. (2001). Uncertainty in estimating blood ethanol concentrations by analysis of vitreous humour. *Journal of Clinical Pathology*, *54*, 699-702.

McCoy, M. A. (2004). Hypomagnesaemia and new data on vitreous humour magnesium concentration as a postmortem marker in ruminants. *Magnesium Research*, *17*(2), 137-145.

McLaughlin, B. G., & McLaughlin, P. S. (1988). Equine vitreous humor chemical concentrations: Correlation with serum concentrations and postmortem changes with time and temperature. *Canadian Journal of Veterinary Research*, *52*, 476-480.

Swann, D. A., Constable, I. J., & Caulfield, J. B. (1975). Vitreous structure. IV. Chemical composition of the insoluble residual protein fraction from the rabbit vitreous. *Investigative Ophthalmology*, *14*(8), 613-616.

Varela, R. A., & Bossart, G. D. (2005). Evaluation of biochemical analytes in vitreous humor collected after death in West Indian manatees. *Journal of the American Veterinary Medicine Association*, *226*(1), 88-92.

Wilkie, I. W., & Bellamy, J. E. C. (1982). Estimation of antemortem serum electrolytes and urea concentrations from vitreous humor collected postmortem. *Canadian Journal of Comparative Medicine, 46*(2), 146-149.

Zaugg, J. L., & Kinsel, M. L. (1997). Vitreous humor analysis for selected biochemical parameters from cervids in Idaho. *Journal of Wildlife Disease*, *33*(4), 776-782.